

Supporting Information

Article title: Pre-zygotic mate selection affects progeny fitness and is only partially correlated with the expression of *NaS*-like RNases

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The following Supporting Information is available for this article:

Fig. S1 Quantification of the post-pollination ethylene burst (PPEB) in 650 recombinant inbred lines (RILs) from a 26-parent Multiparent Advanced Generation Inter-Cross (MAGIC) population of *Nicotiana attenuata*.

Fig. S2 Variation in *Nicotiana attenuata* seedling phenotype of different parental genotype combinations.

Fig. S3 Early growth dynamics of *Nicotiana attenuata* seedlings.

Fig. S4 Number of leaves and secondary roots of *Nicotiana attenuata* seedlings 14 days after sowing (DAS).

Fig. S5 Manhattan plots summarizing the results of the Genome Wide Association Study (GWAS) on the post-pollination ethylene burst (PPEB) in *Nicotiana attenuata*.

Table S1 List of primers used in this study

Table S2 Spearman's rank correlation coefficients between the post-pollination ethylene burst (PPEB) and the gene expression and protein abundance of SLR1 and SLR2 in *Nicotiana attenuata*.

Table S3 Genome Wide Association Study (GWAS) results for the SNPs associated with the post-pollination ethylene burst (PPEB) in the *Nicotiana attenuata* 26-parent Multiparent Advanced Generation Inter-Cross (MAGIC) population after pollination with Utah wild type (UtWT) standard pollen donor.

Table S4 Genes selected as candidates for further research after the Genome Wide Association Study (GWAS) on post-pollination ethylene burst (PPEB) in *Nicotiana attenuata*.

Fig. S1 Quantification of the post-pollination ethylene burst (PPEB) in 650 recombinant inbred lines (RILs) from 26-parent Multiparent Advanced Generation Inter-Cross (MAGIC) population of *Nicotiana attenuata*. The PPEB data obtained after single hand pollination of each RIL line with the pollen from Utah wild type accession are shown in grey. Lines selected for further analyses of the correlation between ethylene emission and SLR expression and protein abundance (Fig. 2) are highlighted: lines with the lowest PPEB in blue and lines with the highest PPEB in red. The PPEB varied in range from 37.78 to 801.75 ppbv. The PPEB data obtained after single hand pollination of Utah wild type with the pollen from each RIL line are shown in green. The PPEB varied in range from 22.89 to 881.26 ppbv. Separate ordering of the RILs (x-axis) was performed for each dataset in order to plot PPEB (y-axis).

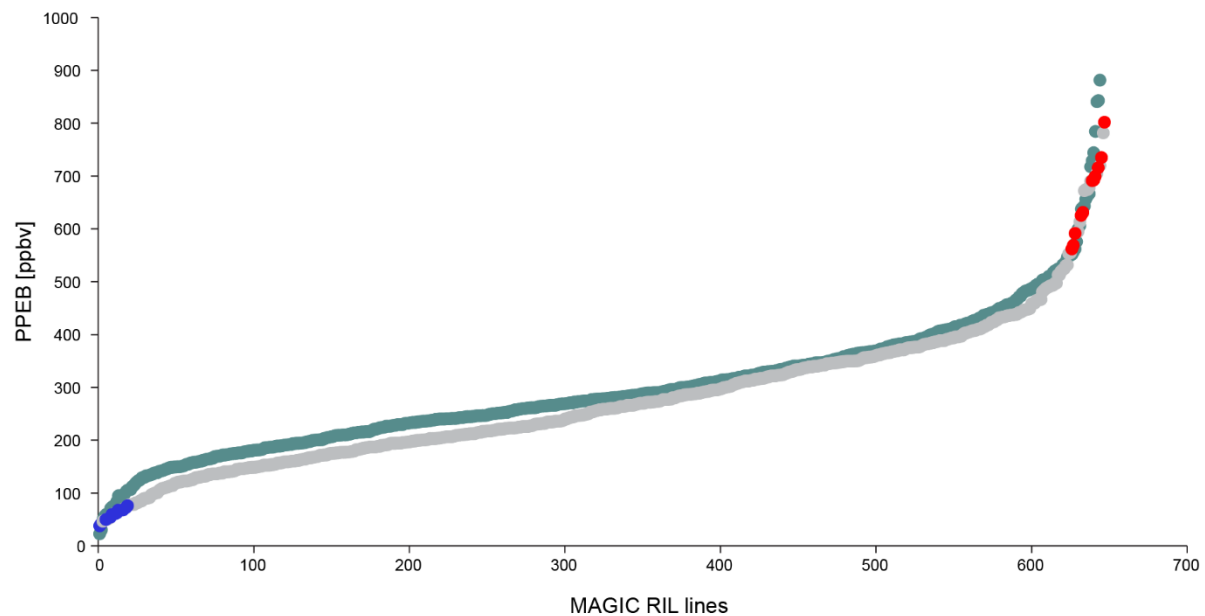


Fig. S2 Variation in *Nicotiana attenuata* seedling phenotype of different parental genotype combinations. Representative seedlings of three different maternal genotypes after the pollination with a favored, non-favored or neutral pollen donor photographed 14 days after sowing, before the harvest.

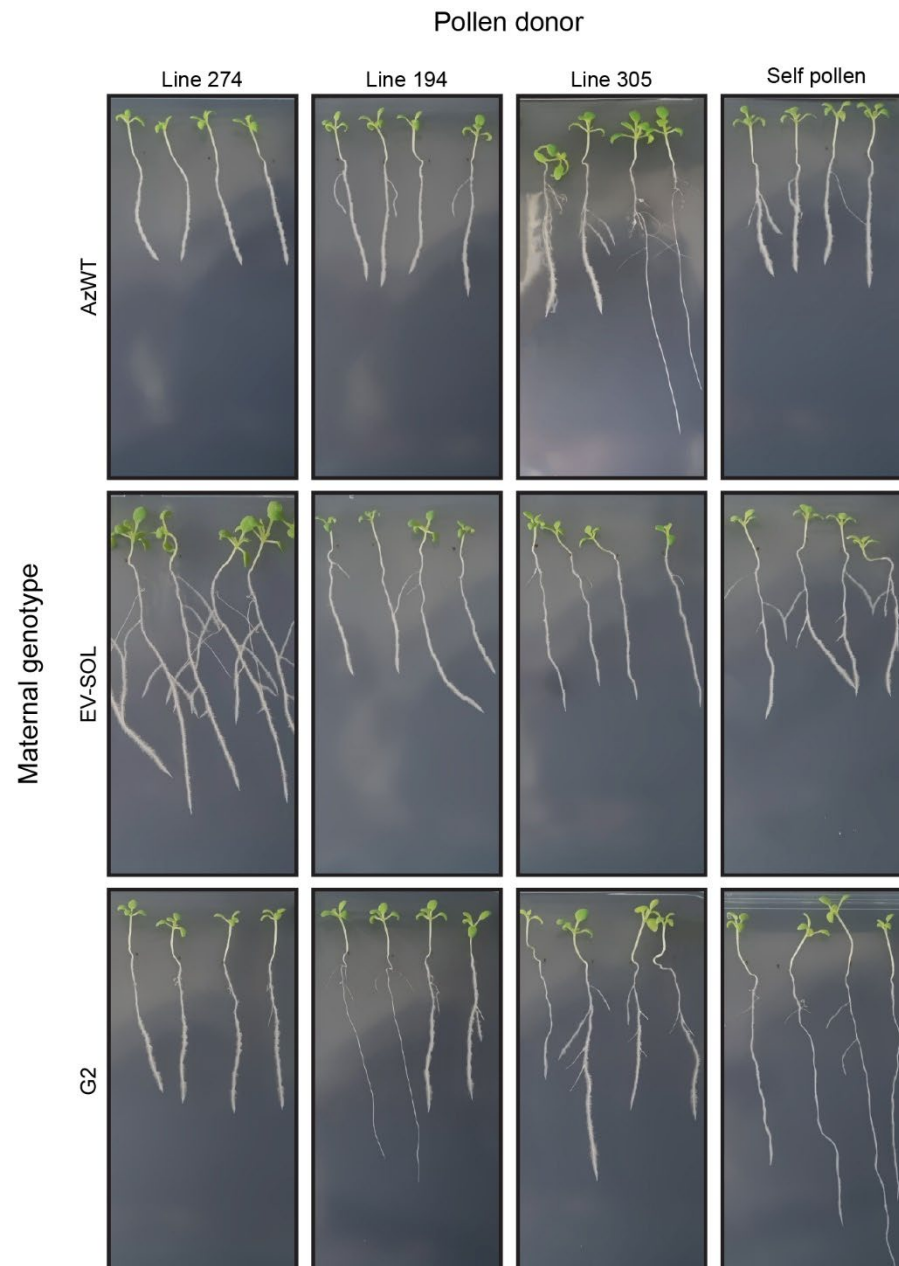


Fig. S3 Early growth dynamics of *Nicotiana attenuata* seedlings. The early growth dynamics were determined for the offspring of different maternal genotypes after single hand pollination with a favored, non-favored, neutral or self pollen donor. (a) Root length (the length of tap root), (b) root area (the sum of lengths of tap root and secondary roots), (c) hypocotyl length, and (d) rosette area were measured from scans of the seedlings using ImageJ. The seedlings were scanned every second day starting 4 days after sowing (DAS) for 14 days. Data are shown as means of up to 30 seedlings per cross - 10 randomly selected seeds from the three different capsules obtained from the single pollination experiment (related data in Figs 3-5). Statistical differences were calculated 14 DAS using mixed effect models with a Sidak *post hoc* test to extract multiple comparisons; small letters (below the line) indicate statistical differences dependent on the pollen donor, $P < 0.05$.

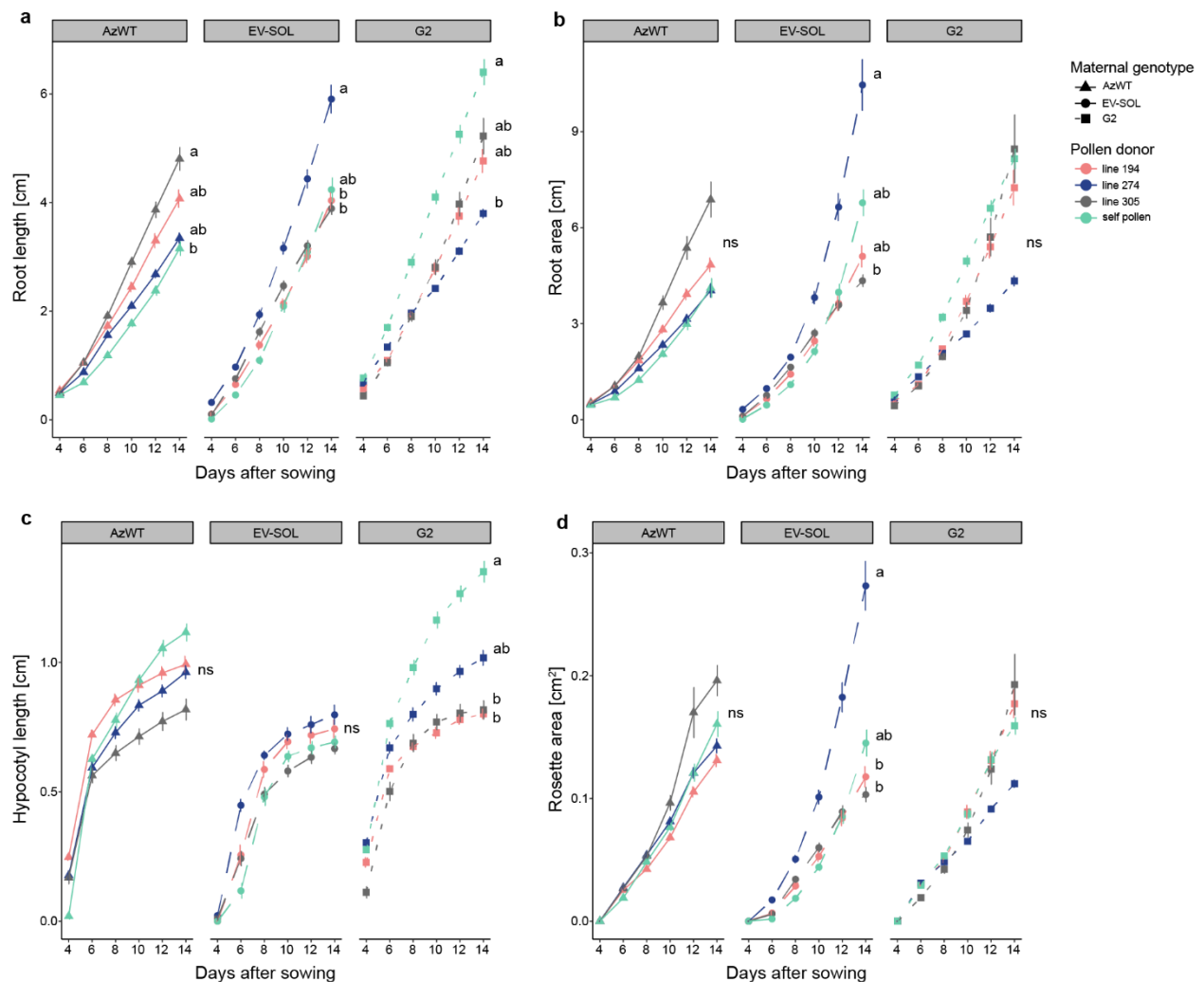


Fig. S4 Number of leaves and secondary roots of *Nicotiana attenuata* seedlings 14 days after sowing (DAS). (a) Number of leaves and (b) lateral roots were determined for the offspring of different maternal genotypes after single hand pollination with a favored, non-favored, neutral or self pollen donor. The seedlings were scanned every second day starting 4 DAS for 14 days and the scans were used to extract the data.

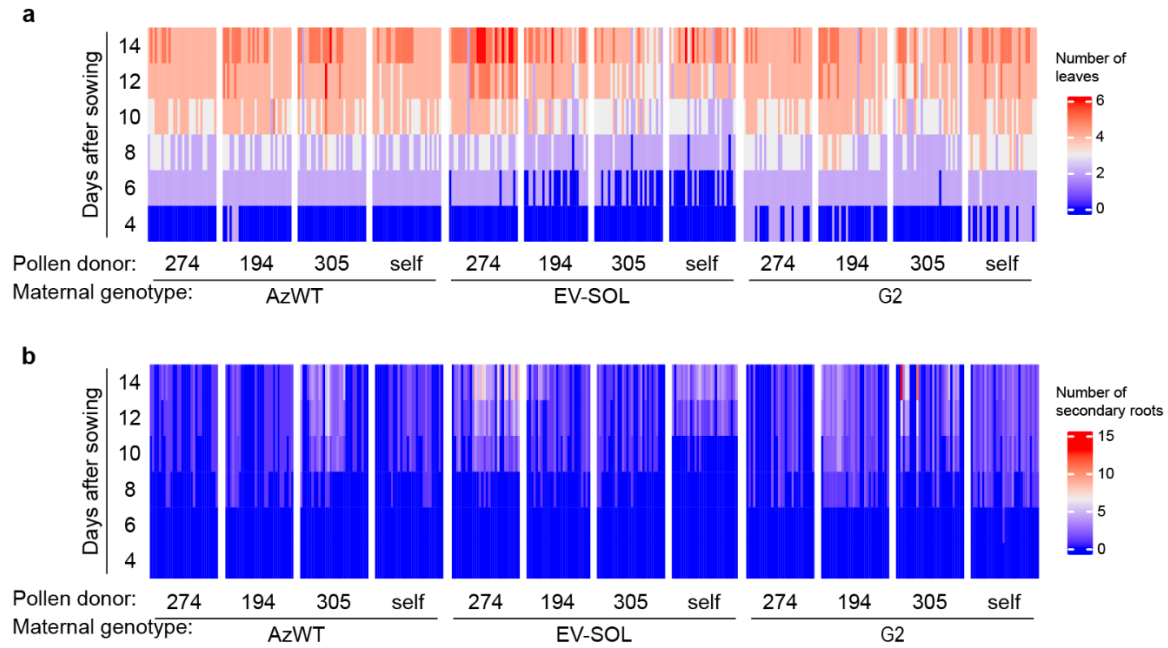


Fig. S5 Manhattan plots summarizing the results of the Genome Wide Association Study (GWAS) on the post-pollination ethylene burst (PPEB) in *Nicotiana attenuata*. The results of the GWAS on PPEB obtained after reciprocal the pollination of (a) different maternal genotypes (629 RIL lines from the MAGIC population) with a Utah wild type (UtWT) standard pollen donor and (b) the standard maternal genotype (UtWT) with different pollen donors (629 RIL lines from the MAGIC population). The strongest association peak on chromosome 9 is shown in blue. Additional significantly associated markers are shown in red. The horizontal dashed line indicates the significance threshold set to 1e-05. The x-axis indicates the position of each SNP in the genome and the y-axis shows the negative logarithm of the p-value obtained from the GWAS model. Different linkage groups are shown in different colors. Scf – scaffold of the genome assembly.

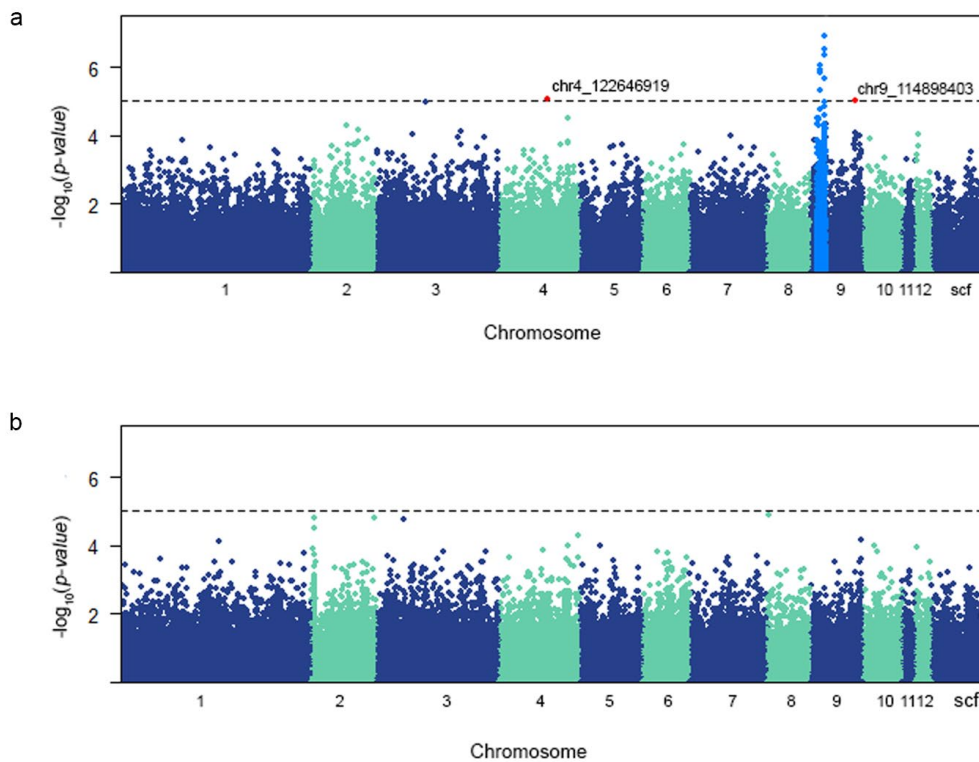


Table S1 List of primers used in this study

Gene	Primer name	Sequence (5' to 3')
<i>Na</i> SLR1	SLR1-RT-F	CACATTTAACGAAACCAGAGATGCC
<i>Na</i> SLR1	SLR1-RT-R	TTTATATGTTTCGACGCACTTGAGGT
<i>Na</i> SLR2	SLR2-RT-F	AGCGTATCCTAACCTCAATTGCATT
<i>Na</i> SLR2	SLR2-RT-R	GTTGCCTCTCGGTTGAAACAGA
<i>Na</i> EF1a	EF1a-F	CCACACCTCCCACATTGCTGTCA
<i>Na</i> EF1a	EF1a-R	CGCATGTCCCTCACAGCAAAAC

Table S2 Spearman's rank correlation coefficients between the post-pollination ethylene burst (PPEB) and the gene expression and protein abundance of SLR1 and SLR2 in *Nicotiana attenuata*. Significant correlations are underlined.

	PPEB : SLR1		PPEB : SLR2	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Relative expression	0.13	0.19	<u>-0.38</u>	<u>4.1e-05</u>
Protein abundance	-0.026	0.79	<u>-0.38</u>	<u>6.6e-05</u>

Table S3 Genome Wide Association Study (GWAS) results for the SNPs associated with post-pollination ethylene burst (PPEB) in the *Nicotiana attenuata* 26-parent Multiparent Advanced Generation Inter-Cross (MAGIC) population after pollination with Utah wild type (UtWT) standard pollen donor. The table provides the SNP ID (SNP), linkage group (Chromosome), base pair position of the marker (Position), p-value (*P*), minor allele frequency (MAF), the amount of variation explained by each marker (R^2), p-value following the procedure controlling the false discovery rate (FDR) and the allelic effect estimate (Allelic effect). Sample size = 629. The significant associations were declared based on an LOD threshold of 1e-05 and are shown above the dashed line. Additional SNPs associated with the peak on chromosome 9 which were used for the selection of candidate genes are shown below the dashed line.

SNP	Chromosome	Position	<i>P</i>	MAF	<i>R</i> ²	FDR	Allelic effect
chr9_33910631	9	33910631	1.19E-07	0.334658188	0.109138598	0.021865296	46.28255771
chr9_34550003	9	34550003	3.15E-07	0.453895072	0.106077878	0.028853351	39.94711141
chr9_33911093	9	33911093	4.71E-07	0.314785374	0.104815634	0.028853351	42.85446266
chr9_23566194	9	23566194	8.65E-07	0.322734499	0.102913666	0.039778506	42.66627007
chr9_23566261	9	23566261	1.16E-06	0.321144674	0.102003536	0.042618002	42.27956624
chr9_24668222	9	24668222	1.46E-06	0.355325914	0.101280458	0.044808013	38.93679848
chr9_34639866	9	34639866	2.08E-06	0.466613672	0.100180575	0.054731257	-36.30640923
chr9_24958799	9	24958799	4.83E-06	0.461844197	0.097578978	0.111043997	-31.42123162
chr4_122646919	4	122646919	8.39E-06	0.320349762	0.095877018	0.160358583	40.39846815
chr9_114898403	9	114898403	9.90E-06	0.235294118	0.095369509	0.160358583	-38.09307256
chr9_34540947	9	34540947	1.04E-05	0.470588235	0.095231257	0.160358583	33.78920023
chr9_34527745	9	34527745	1.44E-05	0.471383148	0.094228425	0.203311507	33.37371145
chr9_22742053	9	22742053	1.75E-05	0.368044515	0.093627995	0.229738193	-38.32508298
chr9_34538085	9	34538085	2.59E-05	0.46581876	0.092427596	0.300710507	30.58005968
chr9_34641035	9	34641035	2.62E-05	0.466613672	0.092399026	0.300710507	31.71154558
chr9_15468213	9	15468213	3.07E-05	0.321144674	0.091915527	0.312560159	37.48807796
chr9_20664570	9	20664570	3.08E-05	0.417329094	0.091901099	0.312560159	-33.03842207
chr9_20251720	9	20251720	3.40E-05	0.353736089	0.091602273	0.312560159	34.78062146
chr9_37752304	9	37752304	4.66E-05	0.390302067	0.090646102	0.36831735	32.08830099
chr9_33910755	9	33910755	4.66E-05	0.352941176	0.090643397	0.36831735	33.10267059
chr9_14312849	9	14312849	4.74E-05	0.309220986	0.090591666	0.36831735	36.5187105
chr9_34550760	9	34550760	4.97E-05	0.475357711	0.090448076	0.36831735	29.75647313
chr9_21530138	9	21530138	5.27E-05	0.461844197	0.09026941	0.373028753	31.34252268
chr9_34552619	9	34552619	5.74E-05	0.476947536	0.090012474	0.385315009	29.51279708
chr9_33910882	9	33910882	5.87E-05	0.321144674	0.089946942	0.385315009	32.23052847
chr9_34530934	9	34530934	6.42E-05	0.468203498	0.089674598	0.398213169	28.88884572
chr9_36956308	9	36956308	6.49E-05	0.388712242	0.089638667	0.398213169	32.0423806
chr9_34615976	9	34615976	7.51E-05	0.457074722	0.089198218	0.431914842	29.11170309
chr9_34945282	9	34945282	8.66E-05	0.362480127	0.088769985	0.439198259	33.18234032
chr9_34648379	9	34648379	8.72E-05	0.472972973	0.088747846	0.439198259	29.61976025

Table S4 Genes selected as candidates for further research after the Genome Wide Association Study (GWAS) on post-pollination ethylene burst (PPEB) in *Nicotiana attenuata*. The table provides the gene code (GOI), gene name and annotation, the position of the gene on chromosome 9, the most significantly associated marker and its p-value. LOD threshold = 1e-05.

GOI	Name	Annotation	Position		Marker	P
			Start	End		
Niat3g_65650	ERF061	Ethylene-responsive transcription factor 061	25738069	25738948	chr9_23566194	8.65E-07
Niat3g_66018	LRR1	Putative LRR protein kinase	35002162	35006145	chr9_33910631	1.19E-07
Niat3g_65722	LRR2	Putative LRR protein kinase	26738712	26742144	chr9_24668222	1.46E-06
Niat3g_65615	ENL3	Early nodulin-like protein 3	24287027	24288294	chr9_23566194	8.65E-07
Niat3g_65293	RNT1a	Regulator of nonsense transcripts 1-like protein	17322262	17324573	chr9_15468213	3.07E-05
Niat3g_65295	RNT1b	Regulator of nonsense transcripts 1-like protein	17326468	17329564	chr9_15468213	3.07E-05
Niat3g_65296	RNT1c	Regulator of nonsense transcripts 1-like protein	17329634	17332325	chr9_15468213	3.07E-05